030

Construction of ubiquitin variance

Prepared versions

Version 1.0 ⟶ A only
Version 2.0 ⟶ B only
Version 3.0 ⟶ A, B are overlapping
Version 4.0 — no A, B

Version
oligos ... A8333C11
A8333C10
diluted to 100 pmol/µl
50 µl each mixed
↓ 94 – 40 slow cool

Ligation — 7 µl oligos (100 pmol)
2 µl 42K DNA
7 µl H_2O
1.5 µl 10X buffer
1.5 µl 10mM ATP
1.0 µl ligase
15 µl
↓ 16° 1 hr.
↓ 65°C 15'

[redacted]

[illegible signatures]

PromGene Promoters

Name	Description	Reporter	Test vector	Transform	Events
PGNpr1	maize polyubiquitin 1 (UBI1)	GUS	pPHP8904	Corn	GSA
		GUS-6xhis	pPGN7062	Corn	GSB
PGNpr2	maize globulin 1	GUS-6xhis	pPGN9005	Corn	
PGNpr3	maize 27 kD alpha-zein	GUS-6xhis	pPGN9001	Corn	PMD
PGNpr4	maize UBI1 no heat shock elements (HSE): UbiC	GUS-6xhis	pPGN7547	Corn	GSC
PGNpr5	maize UBI1 no 3' HSE: UbiD	GUS-6xhis	pPGN7565	Corn	GSD
PGNpr6	maize UBI1 no 5' HSE: UbiE	GUS-6xhis	pPGN7583	Corn	GSE
PGNpr7	maize UBI1 no HSE overlap: UbiF	GUS-6xhis	pPGN7600	Corn	GSF
PGNpr8	maize UBI1 replace HSE with 3x Ps1 seed specific element: UbiG	GUS-6xhis	pPGN8926	Corn	GSG
PGNpr9	teosinte polyubiquitin 1	GUS-6xhis	pPGN8984	Corn	GSI
PGNpr10	teosinte polyubiquitin 1a	GUS-6xhis	pPGN8985	Corn	GSM
PGNpr11	sorghum polyubiquitin 1	GUS-6xhis	pPGN8986	Corn	GSN
PGNpr12	maize glutathione-S-transferase 1 (GST1)	GUS-6xhis	pPGN8987	Corn	GSP
PGNpr13	synthetic promoter RsynD with 35S enhancer 5' (tested with maize Adh-1 intr	GUS-6xhis	pPGN9006	Corn	GSR
PGNpr14	synthetic promoter Rsyn7 with 35S enhancer 5' (tested with maize Adh-1 intr	GUS-6xhis	pPGN9007	Corn	GSS
PGNpr15	maize HRGP	GUS-6xhis	pPGN9016	Corn	SCA
PGNpr16	maize P promoter (tested with maize Adh-1 intron)	GUS-6xhis	pPGN9035	Corn	SCD
PGNpr17	modified version of Agro mannopine synthase (superMAS)	GUS	pPHP10336	Pea	GSH
PGNpr18	bean phaseolin	GUS-10xasp	pPGN3275	Pea	GSJ
		GUS-6xhis	pPGN5690	Pea	GSK
PGNpr19	maize UBI1 no 5' HSE with a little extended seq at 5' end (beyond pt6)	GUS-6xhis	pPGN9042	Corn	GST
PGNpr20	rice glutelin (1.3Kb of 5' sequence)	GUS-6xhis	pPGN9056	Corn	PMA
PGNpr21	rice glutelin 2.3Kb (2.3Kb of 5' sequence)	GUS-6xhis	pPGN9057	Corn	PMB
PGNpr22	rice globulin 26kDa	GUS-6xhis	pPGN9060	Corn	PMC
PGNpr23	maize globulin 2	GUS-6xhis	pPGN9076	Corn	

GIBCO BRL Custom Primers

Certificate of Analysis

Primer 1:

Primer Name: UBI HSP VER. 1A
Researcher:
Primer Number: A8333C10 (C10)
Primer Length: 66
Sequence (5' to 3'): PAG ACG GCA CGG CAT CTC TGT CGC TGC CTC CAC CGT TGG ACT TGC TCC GCT GTC GGC ATC CAG AAA T

Molecular Weight µg/µmole:	21299.2	µg per OD:	31.3
Millimolar Extinction Coefficient:	678.6	nmoles per OD:	1.4
Purity	Desalted	OD's	39.3
Tm (1 M Na+)	96	µg's*	1234
Tm (50 mM Na+)	76	nmoles	57
% GC	60	Coupling Eff.	99%
Notes:			

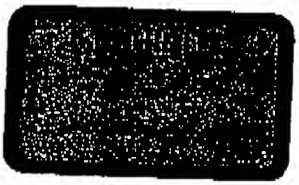
Primer 2:

Primer Name: UBI HSP VER.1B
Researcher:
Primer Number: A8333C11 (C11)
Primer Length: 67
Sequence (5' to 3'): PTT TCT GGA TGC CGA CAG CGG AGC AAG TCC AAC GGT GGA GGC AGC GAC AGA GAT GCC GTG CCG TCT GC

Molecular Weight µg/µmole:	21697.4	µg per OD:	29.8
Millimolar Extinction Coefficient:	732.9	nmoles per OD:	1.3
Purity	Desalted	OD's	10.7
Tm (1 M Na+)	97	µg's*	319
Tm (50 mM Na+)	78	nmoles	14
% GC	62	Coupling Eff.	98%
Notes:			

UBI 1A 57 nmoles 570 µl → 100 pmol/µl

14 nmoles 140 µl → 100 pmol/µl



*-See Note about Quantities in Supporting Information.

LIFE TECHNOLOGIES.

GIBCO BRL Custom Primers
Certificate of Analysis

Primer 1:

Primer Name: UBI HSPA VER.2A
Researcher:
Primer Number: D0373B07 (B07)
Primer Length: 61
Sequence (5' to 3'):P-A GAC GGC ACG GCA TCT CTG TCG CTG CCT CTG GAC CCC TCT CGA CCA CCG TTG GAC TTG CTC CGC TGT CGG CAT CCA GAA AT

Molecular Weight µg/µmole:	26105.2		
Millimolar Extinction Coefficient:	824.3	µg per OD:	31.8
		nmoles per OD:	1.2
Purity	Desalt		
Tm (1 M Na+)	96	OD's	90.0
Tm (50 mM Na+)	77	µg's*	2850
% GC	61	nmoles	108
Notes:		Coupling Eff.	98%

Primer 2:

Primer Name: UBI HSPB VER.2B
Researcher:
Primer Number: D0373B08 (B08)
Primer Length: 82
Sequence (5' to 3'):P-T TTC TGG ATG CCG ACA GCG GAG CAA GTC CAA CGG TGG TCG AGA GGG GTC CAG AGG CAG CGA CAG AGA TGC CGT GCC GTC TGC

Molecular Weight µg/µmole:	26872.4		
Millimolar Extinction Coefficient:	902.2	µg per OD:	29.7
		nmoles per OD:	1.1
Purity	Desalt		
Tm (1 M Na+)	99	OD's	77.0
Tm (50 mM Na+)	78	µg's*	2294
% GC	63	nmoles	86
Notes:		Coupling Eff.	98%

Primer 3:

Primer Name: UBI HSPA VER.3A
Researcher:
Primer Number: D0373B09 (B09)
Primer Length: 81
Sequence (5' to 3'):P-A GAC GGC ACG GCA TCT CTG TCG CTG CCT CTC GAG AGT TCC GCT CCA CCG TTG GAC TTG CTC CGC TGT CGG CAT CCA GAA AT

Molecular Weight µg/µmole:	25160.2		
Millimolar Extinction Coefficient:	830.6	µg per OD:	31.5
		nmoles per OD:	1.2
Purity	Desalt		
Tm (1 M Na+)	98	OD's	88.7
Tm (50 mM Na+)	76	µg's*	2793
% GC	60	nmoles	106
Notes:		Coupling Eff.	98%

*-See Note about Quantities in Supporting Information.

[redacted]

LIFE TECHNOLOGIES

GIBCO BRL Custom Primers
Certificate of Analysis

Primer 4:

Primer Name: UBI HSPB VER.3B
Researcher:
Primer Number: D0373B10 (B10)
Primer Length: 82
Sequence (5' to 3'):P-T TTC TGG ATG CCG ACA GCG GAG CAA GTC CAA CGG TGG AGC GGA ACT CTC GAG AGG CAG CGA CAG AGA TGC CGT GCC GTC TGC

Molecular Weight µg/µmole:	25516.4	µg per OD:	29.7
Millimolar Extinction Coefficient:	901.3	nmoles per OD:	1.1
Purity	Desalt	OD's	83.2
Tm (1 M Na+)	99	µg's*	2478
Tm (50 mM Na+)	77	nmoles	92
% GC	62	Coupling Eff.	98%
Notes:			

Primer 5:

Primer Name: UBI HSPA VER.4A
Researcher:
Primer Number: D0373B11 (B11)
Primer Length: 96
Sequence (5' to 3'):P-A GAC GGC ACG GCA TCT CTG TCG CTG CCT CTG GAC CGC TCT CGA CTC GAG AGT TCC GCT CCA CCG TTG GAC TTG CTC CGC TGT CGG CAT CCA GAA AT

Molecular Weight µg/µmole:	30988.2	µg per OD:	31.7
Millimolar Extinction Coefficient:	976.3	nmoles per OD:	1.0
Purity	Desalt	OD's	89.3
Tm (1 M Na+)	100	µg's*	2833
Tm (50 mM Na+)	78	nmoles	91
% GC	61	Coupling Eff.	98%
Notes:			

Primer 6:

Primer Name: UBI HSPB VER.4B
Researcher:
Primer Number: D0373B12 (B12)
Primer Length: 97
Sequence (5' to 3'):P-T TTC TGG ATG CCG ACA GCG GAG CAA GTC CAA CGG TGG AGC GGA ACT CTC GAG TCG AGA CGG GTC GAG AGG CAG CGA CAG AGA TGC CGT GCC GTC TGC

Molecular Weight µg/µmole:	31791.4	µg per OD:	29.6
Millimolar Extinction Coefficient:	1070.6	nmoles per OD:	0.9
Purity	Desalt	OD's	97.1
Tm (1 M Na+)	100	µg's*	2883
Tm (50 mM Na+)	79	nmoles	90
% GC	62	Coupling Eff.	98%
Notes:			

* -See Note about Quantities in Supporting Information.

[redacted]

LIFE TECHNOLOGIES

9-007

Do Nucleobond preps of 5596, 5597,
4216, 4217, 4218 and 4219

→ Digest 5596 and 5597 w/ EcoRI as a check
make sure smaller frag. is ~2kb.

Digest 4216, 4217, 4218, 4219 w/ BglII/SalI
to check that the 168 bp frag is generated

Digest 4216 w/ BspEI/XcmI to use as
accepting vector for ubiquitin versions 1-4.
Gel isolate on 1% agarose

Digest 5596 w/ NheI/NotI to isolate insert
(~1.6 kb) Z4:BAASS. NotI and Nhe
Gel isolate on agarose cut in different buffers
Cut w/ NotI 1st Ø [illegible]
and ZI-CH ppt.
Cut w/ NheI

Mini-preps on BAASS:M
#s 5648 - 5665
Digest w/ EcoRI/PstI. Cut 1/8 also
Run on 10% acrylamide

→ Anneal Ubiquitin versions 1, 2, 3, and 4
oligos together.
Heat to 95°C for 5 min then stick on ice
check these on a 10% acrylamide gel

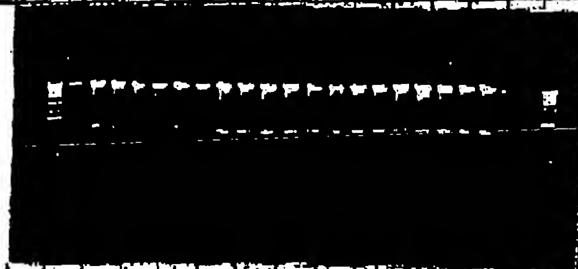
Run precut 3770 NheI/NotI on an agarose
gel to check it out [illegible signature]

[redacted]

13-004

Check Ubi variation w/ a H3/Not I digest (first 5 of each)

1 1Kb ladder
2-6 Ubi 1 7593-7597
7-11 Ubi 2 7561-7565
12-16 Ubi 3 7579-7583
17-21 Ubi 4 7597-7601
22 [illegible] NL/NotI 7062 vector
23 6439 Nco/NotI frag
24 1Kb ladder



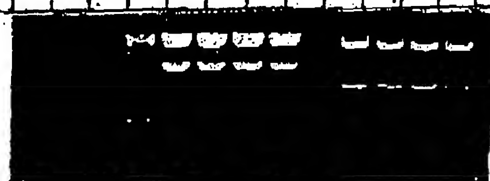
All look fine Send these for sequencing

Sequencing data shows 7368 to be correct for GUS/RZP

Make Avidin/RZP using 7368 construct

Digest 7424 (Avidin/3770) and 7368 (GUS/RZP) with BamHI/XbaI

Gel: 1 1Kb ladder
2-5 7368 (GUS/RZP) Bam/Xba
6 Skip
7-10 7424 (Avidin) Bam/Xba



Isolate large vector frag from GUS/RZP and the smaller insert band from Avidin

Katherine [signature]

[redacted]

Gus Assay

PURPOSE: TO QUANTITATE THE AMOUNT OF GUS IN CORN SEED

MATERIALS: READING PLATE - COSTAR EIA/RIA
READING PLATE - NUNC. FLUORONUNC POLYSORP
MU - 4 METHYLUMBELLIFERONE (SIGMA M-1508)
MUG - 4 METHYLUMBELLIFERONE β GLUCURONIDE (SIGMA M-9130)
MICROBALANCE
FLUORESCENCE MICROPLATE READER

PROCEDURE: USE PROTOCOL FOUND ON PAGE* 57 OF THIS NOTEBOOK (#58).

RESULTS: DATA FOUND BELOW. (BASED ON 20-MIN READINGS.

SAMPLE#	%TSP	SAMPLE#	%TSP
GSE 12020-4	.088	GSE 06030-1	0.087
-5	ND	-2	0.54
GSD 01120-1	ND	-3	0.61
-2	ND	-4	0.16
-3	ND	-5	0.06
-4	ND	" 0828-1	0.031
-5	ND	-2	0.002
GSE 15070-4	0.28	-3	0.027
" 050501	0.17	-4	ND
-2	0.015	-5	0.001
-3	0.010	" 07050-1	0.3
-4	0.174	-2	0.089
-5	0.010	-3	0.27
" 05090-1	0.043	-4	0.013
-2	0.014	-5	0.43
-3	0.001		
-4	0.004		
-5	0.004		
GSC 01010-1	0.008		
-2	0.010		
-3	0.009		
-4	0.60		
-5	~~ND~~ 0.48		

[redacted]

Investigator: Book # 58

Chris Brook Date:

Witness: Elizabeth Wilfong Date:

GUS ASSAY

SEE PURPOSE, MATERIALS, & PROCEDURE BELOW.

Purpose: To quantitate the amount of GUS in corn seed extracts.

Materials: Reaction Plate-Costar EIA/RIA, [illegible] 96-well flat bottom plate
Reading Plate-Nunc Fluorescence Polysorp 96-well black plate
MU 4-methylumbelliferone (Sigma M-1508)
MUG 4-methylumbelliferyl-β-glucuronide (Sigma M-9130)
Microbalance
Fluorescence microplate reader (Molecular Devices Gemini)

Reagents: Lysis Buffer: 50 mM sodium phosphate pH 7.0, 1 mM EDTA, 10 mM BME. [illegible] (null)

Note: 50 mM sodium phosphate is made by mixing 97 ml of Stock A (0.2M NaH_2PO_4 (27.6 g/L)) with 153 ml of Stock B (0.2M Na_2HPO_4 (53.6 g/L)) and bringing to a final volume of 1.0 L with dH_2O.
Also note that the 10 mM BME should be added to an aliquot of the lysis buffer fresh daily, enough for that day's experiment.

Stop Buffer: 0.2 M Na_2CO_3 (21.2 g/L)
1 mM MU Standard Stock: 4.96 mg MU in 25 ml dH_2O (made fresh daily)
20 mM MUG Substrate Stock: 7 mg MUG in 1.0 ml 95% ethanol (made fresh daily).

Procedure: Corn seed extracts should already be prepared and analyzed for total protein according to standard procedures.

In a reaction plate, equilibrate up to 10 µg of total protein in a total volume of 100 µl lysis buffer. Generally samples can be analyzed with 1 µg total protein. Samples should be analyzed in triplicate. (lands long)

Add standard curve to triplicate wells diluted as follows:

10 µl of 1 mM MU standard stock is diluted with 90 µl lysis buffer.
10 µl of this 1:10 dilution is further diluted with 90 µl lysis buffer to give a 1:100 dilution.

0 nM MU standard	100 µl lysis buffer / well
1000 nM MU standard	12.5 µl of the 1:100 dilution + 87.5 µl lysis buffer / well
10,000 nM MU standard	12.5 µl of the 1:10 dilution + 87.5 µl lysis buffer / well
100,000 nM MU standard	12.5 µl of the 1 mM MU stock + 87.5 µl lysis buffer / well

Prepare the reading plates by pipetting 175 µl of Stop buffer into all wells of the plate. You will need a separate plate for each time point required. Generally we take readings at 0, 15, 30 and 60 minutes.

Dilute the 20 mM MUG substrate stock to 5 mM with lysis buffer. Add 25 µl of 5 mM MUG to every well including both standard and sample wells and mix to start the reaction. Immediately after adding the MUG, pipette 25 µl of solution from the reaction plate into a prepared reading plate. Place the reaction plate at 37 °C until the next time point. At each subsequent time point, pipette 25 µl of solution from the reaction plate into a prepared reading plate.
Reaction is stable for several hours once it has been stopped. Note that stopping the reaction is essential for fluorescence formation.

Plates are read at 365 nm excitation wavelength and 460 nm emission wavelength.

The unknown samples are read against the standard curve in nM MU and the amount of GUS in the samples is calculated as follows:

Average nM MU for each sample (Mean Value Column)/ minutes reaction proceeded = nM MU / min * 60 min / hr = nM MU / hr. Note that if there is > 100 FU in the 0 timepoint reading of the average nM MU, that value must be subtracted from the average nM MU at each subsequent reading. This value is then corrected for the amount of protein added to the sample by dividing by the total protein added to give nM MU / hr / µg. This value is converted to %TSP by multiplying by $1.88 \times 10^{-?}$ which is a conversion factor determined while at Pioneer.

A Quality Control sample (a known amount of GUS spiked into control corn seed extract) may be run on each assay to determine reproducibility of quantitation.

CB

[redacted]

RESULTS: DATA FOUND BELOW. (10-MIN READINGS)

SAMPLE #	% TSP
GSG 01040-1	~~0~~ 0
-2	~~0.4~~ 0.04
-3	~~0.6~~ 0.06
-4	~~0.5~~ 0.05
-5	~~0.4~~ 0.04
GSD 02130-1	~~1.1~~ 0.1
-2	~~0.7~~ 0.07
-3	~~0.9~~ 0.1
-4	~~0~~ 0
-5	~~0.8~~ 0.1
GSG 01020-1	~~0~~ 0
-2	~~0~~ 0
-3	~~0.12~~ 0.01
-4	~~0~~ 0
-5	~~0.2~~ 0.02
GSC 01030-1	~~0~~ 0
-2	~~4.0~~ 0.4
-3	~~4.2~~ 0.4
-4	~~0.5~~ 0.05
-5	~~4.5~~ 0.5

SAMPLE #	% TSP
GSC 01110-1	~~0.6~~ 0.06
-2	~~0.4~~ 0.04
-3	~~0~~ 0
-4	~~0.4~~ 0.04
-5	~~0.4~~ 0.04
GSC 01070-1	~~4.2~~ 0.4
-2	~~2.7~~ 0.3
-3	~~3.4~~ 0.3
-4	~~5.2~~ 0.5
-5	~~0.01~~ ~~.008~~ 0.001
GSC 01040-1	~~0.1~~ 0.01
-2	~~5.1~~ 0.5
-3	~~0.3~~ 0.03
-4	~~0.3~~ 0.03
-5	~~0.04~~ 0.004

SAMPLE #	% TSP
GSC 01060-1	~~0~~ 0
-2	~~0~~ 0
-3	~~0~~ 0
-4	~~0~~ 0
-5	~~4.8~~ 0.5
GSC 01130-1	~~8.4~~ 0.8
-2	~~0.1~~ 0.01
-3	~~8.6~~ 0.9
-4	~~5.0~~ 0.5
-5	~~0.7~~ 0.07
GSC 01110-1	~~0~~ 0
-2	~~9.2~~ 0.9
-3	~~0~~ 0
-4	~~0~~ 0
-5	~~4.6~~ 0.7

Investigator: [signature] Book # 67

Date:

Witness: [signature] Date: